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Hypoglycemic Effect of the Ethyl-Acetate and Butanol Fractions of *Mallotus oppostifollous* in Mice

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Abstract

A Background: *Mallotus oppostifollous* is a shrub that has historically been used to cure various ailments including infections, wounds, inflammations, and illnesses linked to oxidative stress in places they occur like South East Nigeria. In the present study the ethyl-acetate and butanol fractions of *Mallotus oppostifollous* was evaluated for the hypoglycemic and ant hyperglycemic effect using mice.

Methods: The dried powdered leaf of *M. oppositifolius* was extracted by cold maceration using absolute methanol, filtered and dried using rotary evaporator. The dried extract was partitioned into n-hexane, Ethyl Acetate (EAF), Butanol (BF), and Water Fraction (WF) using standard laboratory procedure. Hyperglycemia was induced by intraperitoneal injection of alloxan monohydrate (120 mg/kg) and the animals were observed for hyperglycemia. A total of 42 hyperglycemic mice with were randomized into 7 experimental groups of six animals per group as follows; group 1 (untreated control), group 2 (hyperglycemic control), group 3 the positive control (metformin, 500 mg/kg) while groups 4 to 7 were administered 250 and 500 mg/kg doses of EAF and BF respectively.

Results: The results showed that the ethyl acetate and butanol fractions contained glycosides, saponins, alkaloids, flavonoids, and tannins. The findings further showed that mice treated with ethyl-acetate and butanol fractions of *Mallotus oppostifollous* had significant reduction ($P<0.05$) in fasting blood glucose levels within 10 hours of acute treatment and further showed significant reduction ($P<0.05$) in fasting blood glucose levels 14 days long term treatment when compared with the hyperglycemic control.

Conclusion: We, therefore, conclude that the hypoglycemic potency of the fractions was greater in the ethyl-acetate fraction than the butanol fraction. Also, the histopathological result showed that mice treated with ethyl-acetate fraction had rejuvenated pancreatic β-cells islets and therefore suggested that ethyl-acetate fraction could contain compounds with known antidiabetic activity. Therefore, we recommend that further study should be carried out to isolate and characterize the compounds in the ethyl-acetate fraction responsible for the active hypoglycemic activity.

Keywords: Phytochemical • Hypoglycemic • *Mallotus oppostifollous* • Butanol fraction • Ethyl-acetate fractions • Blood glucose

Introduction

Globally diabetic mellitus remained a serious public health problem with a huge economic burden and increased mortality. Studies have demonstrated that diabetes is a complex disease whose major symptoms include elevated blood sugar among other symptoms which causes hyperglycemia. Hyperglycemia causes oxidative stress that disrupts regular metabolic activities, resulting to diabetic complications [1,2]. According to a prior study, oxidative stress brought on by hypoglycemia is a major factor in the genesis of diabetes sequelae, including nephropathy [3]. This meant that treating it will involve a variety of therapeutic approaches, including activities that specifically target and stimulates hypoglycemia and antioxidants. A treatment plan that combines both hypoglycemic and antioxidant qualities will be effective for treating and managing diabetes related illness. The major main challenges of the current orthodox chemotherapeutic drugs available for diabetes are their toxicity over time as well as the ineffectiveness of some anti-diabetic medications [4]. As a result, there is a need to look for alternative sources of hypoglycemic medications from the natural sources that are available, free from toxic effect, and effective for the treatment of hyperglycemia. *Mallotus oppositifolius* (Geiseler) Mull Arg. (M. *oppositifolius*) (Euphorbiaceae) is an edible shrub that has a long history of use traditionally among people of South-East Nigeria for the treatment of diseases brought on by bacterial and fungal pathogens as well as for wound healing and eye issues [5].

According to reports, *M. oppositifolius* has antifungal and antibacterial potential against a variety of bacterial infections, including those caused by *E. coli* and *S. aureus* [6,7]. Recent studies reported that the crude extract

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of *M. oppositifolius* showed anti-diabetic [8,9] and antioxidant effect [10]. Previous study also shown that the plant crude extract had no toxic effect when administered at acute dosage and further showed that the ethyl-acetate and butanol had greater antioxidant activity when compared with water and n-hexane fractions and this, therefore, informed our choice of the study because there is a correlation between antioxidants and anti-diabetics activity [11-13]. Reactive Oxygen Species (ROS) are thought to cause diabetes, however, plants that contain antioxidant compounds have been reported to defend β-cells from the activities of Reactive Oxygen Species (ROS) and this implied that antioxidants can therefore prevent diabetes induced by ROS when systematically exploited and therefore, should be exploited and harnessed as a therapeutic biomarker for the treatment of diabetes. Antioxidants with higher potencies and concentrations often alleviate the problems associated with diabetes complications and this is due to their potential to donate electrons to free radicals or perform electron redux action, thereby reducing oxidative stress related to diabetes [14]. This study therefore evaluated the hypoglycemic potential of ethyl-acetate and butanol fractions of *M. oppostifollous*.

Materials and Methods

Apparatus and equipment

The equipment and apparatus includes: Glass column, flasks, beakers, test tubes, measuring cylinders, rotary evaporator, analytic weighing balance (Metler H_3O, Switzerland), a blood glucose meter (One Touch, China), a spectrophotometer (B. Bran Scientific and Instrument Company, England), a water bath (Techmel and Techmel, Texas, USA), a national blender (Japan), and a micropippete (Finnipipette® Labsystems, Finland) are apparatus used for this study [15].

Chemicals, reagents and drugs

The reagents and medications utilized in this research includes: Alloxan monohydrate (Sigma LTD, USA), Metformin, ethanol, ethyl acetate, butanol, (JHD, Guangdong Guanghua Schi-Tech. Ltd China), formaldehyde 40%w/v (May and Baker Ltd, Dagenham, England), and Tween-80 (Sigma Aldrich, Germany).

Animals

A total of 42 white healthy mice that ranged in weight between 25 and 30 grams, purchased were from the laboratory animal facility of the department of veterinary physiology and pharmacology at the University of Nigeria, Nsukka. The mice were then tranferred to the animal house of the department of pharmacology and toxicology at Nnamdi Azikiwe university, where they were used in the experiment. The animals were housed in spotless metal cages, given access to portable water sources, and were fed with commercial pelleted feed (Guniea Feed®, Nigeria).

Collection and authentication of plant materials

Fresh leaves of *Mallotus oppostifollous* were harvested from their natural habitat and were identified and confirmed by a plant taxonomist. To get rid of dust and other debris, the fresh *Mallotus oppostifollous* leaves were washed under running water, dried for two weeks in the open air. *Mallotus oppostifollous* dried leaves were ground using an electrical blender and stored in sterile, airtight amber bottles until needed for extraction.

Extraction of plant material

The pulverized plant material was cold macerated in absolute methanol using the method described [16]. The mixture was regularly stirred for three days (72 hours) to extract properly. Utilizing a water bath set at 40°C, the filtrate was collected and concentrated to dryness.

Fractionation of methanol crude extract

The crude methanol extract was partially purified according to the method using N-hexane, ethyl acetate, and butanol. One hundred grams of crude extract was dispersed in 500 ml of distilled water and then poured inside a separating funnel of 1000 ml capacity. The funnel was then filled with 500 ml of n-hexane, which was properly mixed after being introduced. Two distinct layers of the mixture were allowed to form naturally. After separating the n-hexane component (upper layer), the remaining portion was exposed to new n-hexane solvent until the mixture with n-hexane solvent became transparent. Following the n-hexane phase, the remaining fraction underwent consecutive exposure to ethyl acetate and butanol menstruum using the same procedure as for n-hexane. The various fractions purified were collected and dried using a water bath heated to 40°C.

Phytochemical analysis

According to the steps described, the phytochemical test of the fractions was conducted as follows:

Test for the presence of alkaloids

To about 2 ml of the test, fractions in three test tubes was added 5 drops of Mayers's reagent, Wagner's reagent and picric acid solution (1%) was separately added in different test tubes and observed for colour change and presence of a yellow to orange precipitate.

Test for the presence of tannins

To about 3 ml of the test fractions add 3 drops of ferric chloride. The mixture was observed for a greenish-black precipitate which indicates the presence of tannins. Also, in 3 ml of the fraction in a test tube was added 3 drops of lead acetate solution. The mixture was observed for a brownish colour precipitate indicating the presence of tannins.

Test for the presence of flavonoids

To about Three (3) ml of test fractions were added 2 to 3 drops of diluted ferric chloride solution and observed for deep green color.

Test for the presence of saponins using a frothing test: To about three (3) ml of the test extract was diluted to ten (10) ml with distilled water. The resulting solution was shaken vigorously for a minute and allowed to stand. The mixture was observed for the presence of steady froth (foam), which indicates the presence of saponins.

Test for steroids

Liebermann-Burchard reaction: To about 2 ml of the test fraction was added 2 ml of chloroform and 1-2 ml acetic anhydride plus 2 drops of concentrated H_2SO_4 from the side of test tube. Observe for first red, then blue, and finally green color.

Induction of experimental diabetes

The drug alloxan monohydrate solution was used as a diabetogenic agent for experimental diabetes in mice according to the method described. Forty-two mice of similar age were fasted for 24 hours, followed by injection of a single dose of 120 mg/kg of alloxan monohydrate by intraperitoneal route. The alloxanized mice were kept for 72 hours (3 days) with free access to feed and water for hyperglycaemia to develop. Baseline (zero hours) fasting blood glucose levels were determined using one touch glucometer (Lifescan, USA). Mice with glucose levels above 200 mg/dl were recruited as hyperglycemic mice and used for the study. Note that all the mice induced using the diabetogenic agent was recruited for the study.

Experimental design

Comparative hypoglycemic effect of both fractions of butanol and ethyl-acetate of *Mallotus oppostifollous* as was carried out as described earlier. The 42 hyperglycemic mice were randomized into (6 hyperglycemic) groups of 6 hyperglycemic mice each and one group of 6 non-hyperglycemic mice (an untreated control) as follows:

Animals in group 1 served as untreated control that received only distilled water, group 2 served as negative or hyperglycemic control that received also distilled water, group 3 served as a positive control that received metformin as the standard drug, and groups 4 and 5 were treated with an ethyl-acetate fraction of the extract at 250 and 500 mg/kg

respectively, while groups 6 and 7 were treated with butanol fraction of the extract at 250 and 500 mg/kg respectively.

Blood glucose determination

Daily administration of metformin, and treatment fractions were carried out for a period of 14 days (2 weeks) on the same mice daily. The blood glucose level of the hyperglycemic mice that were treated with the given doses of the 250 and 500 mg/kg of ethyl-acetate and butanol fractions and the positive control respectively were measured at 2, 4, 6, 8, and 10 hours post-treatment using a blood glucose meter.

Blood glucose level of all the test mice were also measured on days 2, 4, 6, 8, 10, 12, and 14 before treatment daily. After administration of the last dose, animals were fasted overnight and the final blood glucose level was taken. Test mice were sacrificed using chloroform anesthesia and the organs were harvested for histology.

Histopathology procedure

The histopathological assay of the harvested organs was carried out according to the methods described.

Tissues harvested from animals were preserved using 10% neutral buffered formalin placed in pre labelled universal containers to ensure each treatment group were represented properly. The tissues were dissected and placed in labeled tissue cassettes using all standard safety operating procedures tissues. The thickness of the dissected tissues sections did not exceed 3-5 mm thickness.

Tissues processing were subjected to an automatic tissue processing using the Leica TP2010 automatic tissue processor for 18 hours passing them through the four stages of tissue processing namely: fixation (using 10% neutral buffered formalin) dehydration (using ascending grades of isopropyl alcohol), clearing or dealcoholizing (using xylene) and finally impregnation or infiltration (using molten paraffin wax).

The tissues were then embedded in paraffin wax using the automated tissue embedder and sectioned to get ultra-thin sections at five (5) microns, using the thermo scientific semi-automated rotary microtome. Tissues were floated out from the thermo scientific digital floating bath on frosted end pre labeled slides and dried on the thermo-scientific digital slimline hot plate.

The processed tissues were further dried in the hot air oven overnight and subjected to hematoxylin and eosin staining to demonstrate the general tissue structure. Stained slides were mounted in DPX and allowed to dry before viewing under the microscope using X 100 and X 400 magnification respectively.

Statistical analysis

The data generated from these assays were analyzed using Statistical Package for Social Sciences (SPSS-20) and the results were presented as mean ± Standard Error of Mean (SEM) of sample replicates.

Results

Phyto-constituents *Mallotus oppostifollous* fractions

The result of the photo-constituents of the ethyl-acetate and butanol fractions of the *Mallotus oppostifollous* is presented in Table 1. It showed the presence of alkaloids, cardiac glycosides, flavonoids, tannins and saponins, while steroids were only present in the ethyl-acetate fraction.

Bioactive compound	Ethyl-acetate	Butanol
Alkaloids	Present+	Present+
Cardiac Glycosides	Present++	Absent
Flavonoids	Present++	Present+
Tannins	Present++	Present+
Saponins	Present++	Present+
Steroids	Present+	Absent

Note: (+) => Present in moderately high concentration, (++) => Present in high concentration.

Table 1. Phytochemicals profile of ethyl-acetate and butanol fraction of *Mallotus oppostifollous*.

Effect of *Maillots oppostifollous* fractions on blood glucose level (acute or hourly study)

The result of effect of the ethyl-acetate and butanol fractions of *Mallotus oppostifollous* on acute treatment (hourly) of hyperglycemia is presented in Figure 1. In each treatment, the fractions significantly (P 0.05) lowered the fasting blood glucose levels in the mice diabetic that had been given alloxan to induce diabetes. Between the first and tenth hours of therapy, the two dosages (250 and 500 mg/kg) of the ethyl-acetate and butanol fractions of *Mallotus oppostifollous* with metformin resulted in a decrease in fasting blood glucose levels. When compared to the untreated group after 2 hours to 10 hours, the dosages of 250 and 500 mg/kg of both the butanol fraction and the ethyl-acetate fraction showed a significant (P 0.05) drop in fasting blood glucose levels. Blood sugar reduction in the fractions was dose-related and substantial (P 0.05). The ethyl acetate fraction provided the best hypoglycemic impact because it had better redeuction in hyperglycemia.

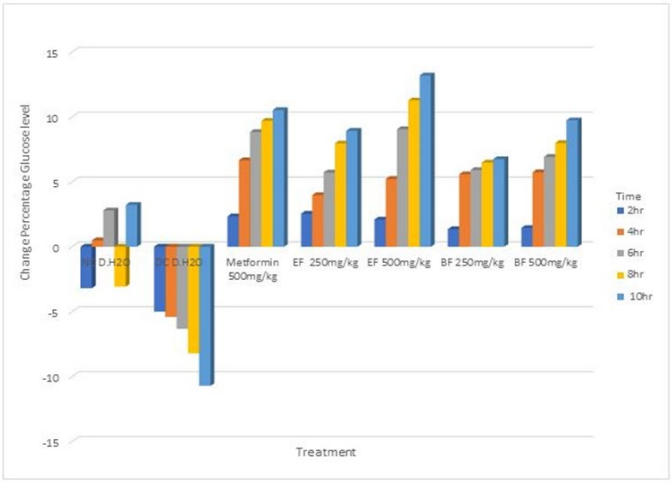


Figure 1. Effect of various treatments on blood glucose level (hourly study).

Note: NC: Untreated control; DC: Diabetic control (hyperglycemic control); EF: Ethyl acetate fraction; BF: Butanol fraction and $D.H_2O$=Distilled water.

Effect of various daily treatment of *Mallotus oppostifollous* Fractions on blood glucose level

The result of the effect of the ethyl-acetate and butanol fractions of *Mallotus oppostifollous* on daily treatment is presented in Figure 2. When compared to the untreated control from day 0 to day 14 of therapy, the two dosages, (250 and 500 mg/kg) of the ethyl acetate fraction and butanol fraction of *Mallotus oppostifollous* as well as positive control (Metformin) produced a significant ($P<0.05$) decrease in the fasting blood glucose levels. The reduction in fasting blood glucose showed a dose dependent reduction. This result also showed that ethyl acetate has the best hypoglycemic effect against alloxan induced hyperglycemia and this is also dose dependent effect [17].

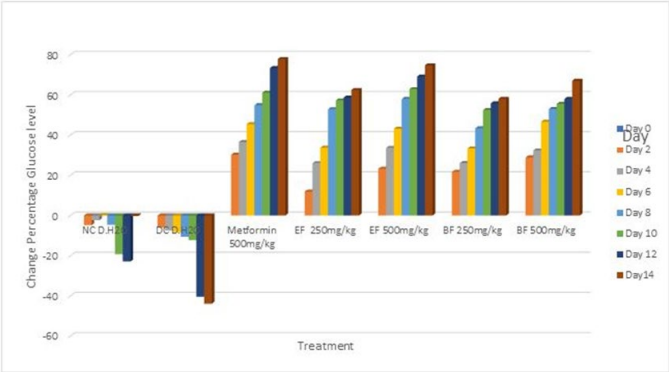
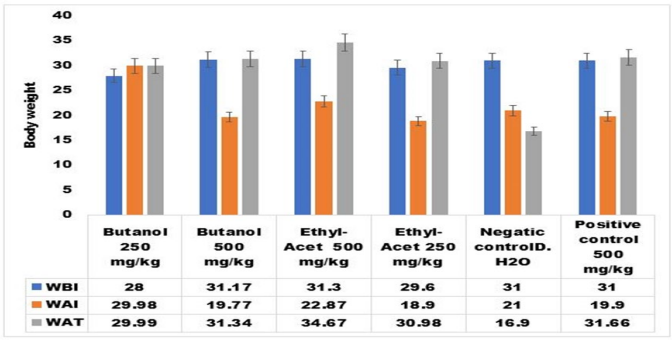


Figure 2. Effect of various treatments on blood glucose level (Daily study).

Note: NC: Untreated control; DC: Diabetic control; EAF: Ethyl acetate; BF: Butanol fraction and D. H_2O: Distilled Water.

Effect of *Mallotus oppostifollous* fractions on body weight of mice

The result of effect of the ethyl-acetate and butanol fractions of *Mallotus oppostifollous* on body weight of mice is presented in Figure 3. The animals' body weight was significantly reduced as a result of the alloxan monohydrate treatment, and this difference from the control group was statistically significant. The outcome, shown in Figure 3, revealed a significant rise in body weight following treatment with different fractions and metformin. When compared to the hyperglycemic control group, weight gain was greater in the normal control and treated groups and this revealed an impressive recovery rate in the mice's body weight for the treated group. Therefore, the mice treated with the *oppositifolius* fractions of butanol and ethyl acetate significantly recovered their body weight, whereas the mice treated with the hyperglycemic (negative) control lost weight and did not recover over the course of it. The group treated ethyl-acetate at 500 mg/kg had the fastest weight recovery rate. The group that received 500 mg/kg of ethyl-acetate treatment experienced the quickest weight recovery.



	Butanol 250 mg/kg	Butanol 500 mg/kg	Ethyl-Acet 500 mg/kg	Ethyl-Acet 250 mg/kg	Negatic controlD. H2O	Positive control 500 mg/kg
WBI	28	31.17	31.3	29.6	31	31
WAI	29.98	19.77	22.87	18.9	21	19.9
WAT	29.99	31.34	34.67	30.98	16.9	31.66

Figure 3. Final Change in body weight of mice in different treatment groups

Note: WBI: Weight before Induction; WAI: Weight after Induction; WAT: Weight After Treatment.

Histopathology examination

The findings of the histopathology examination of the pancreas are presented in plate A to G. The pancreas of the hyperglycemic mice (group 2), without treatment (untreated) were severely degenerated plate B. The photomicrograph of a pancreatic section of mice treated with metformin (plate C) showed normal histoarchitecture with an increase number of islet cells when compared to the hyperglycemic (negative control) group that were severely degenerated (plate B). The photomicrograph of the treatment group 5 and 7 treated with doses 500 mg/kg of butanol (plate G) and ethyl-acetate fractions (plate E) had mild despaired architecture of the pancreatic β cells islet when compared to compared to the hyperglycemic group (negative control) (plate B) that were severely degenerated and this showed a dose dependent anti-diabetic activity of both the ethyl acetate and butanol fractions of *Mallotus oppostifollous*. The untreated group that served as normal control showed normal features of pancreatic cells (Figure 4).

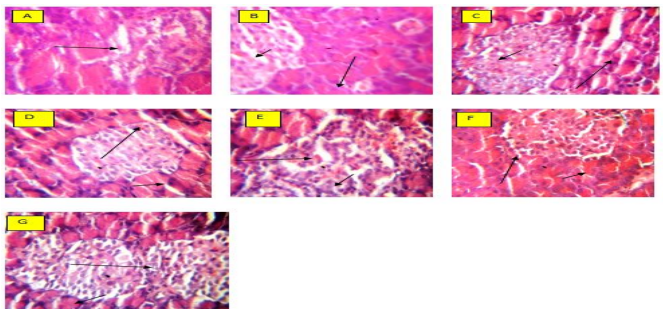


Figure 4. Showing photomicrograph of pancreas histology plate A=Untreated control, plate B=Diabetic control, plate C=Metformin treated, plate D=Ethyl acetate 250 mg/kg treated, plate E=Ethyl acetate 500 mg/kg treated, plate F=Butanol 250 mg/kg treated, plate E=Ethyl acetate 500 mg/kg treated, plate F=Butanol 250 mg/kg treated, plate G=Butanol 500 mg/kg treated.

Discussion

The result showed that the ethyl acetate and butanol fractions of *M. oppostifollous* showed the presence of cardiac glycosides, tannins, saponins, alkaloids, steroids, and flavonoids. These flavonoids have been known to have potent anti-hyperglycemic properties [18]. The presence of these phytochemicals in the butanol and ethyl-acetate fractions, respectively, may be the cause of their hypoglycemic activity since saponins, tannins, and flavonoids are thought to be helpful against diabetes. Previous studies have reported the absence of toxicities in the crude extract *Mallotus oppostifollous* up to 5000 mg/kg oral administration and this suggests that the extract is safe on short-term exposures. As a result, it was demonstrated to be relatively safe at high doses, suggesting that the crude extract is safe for the treatment of hyperglycemia. Additionally, a prior study revealed that the crude methanol extract of *Mallotus oppostifollous* significantly (P 0.05) decreased blood glucose levels from 2 hours to the 10th hour and from day 1 to the 14th of treatment when compared to untreated controls. This is consistent with the results of the current study, which revealed that the butanol and ethyl-acetate fractions both demonstrated dose-dependent hypoglycemic activity [19].

The fractions of *Mallotus oppostifollous* demonstrated a significant ($P<0.05$) decrease in blood glucose level, as this was more pronounced in the high doses (500 mg/kg) for both hourly and daily studies of both ethyl-acetate and butanol fractions respectively. Results demonstrated that the ethyl-acetate fraction significantly lowers blood glucose levels when compared to the control. Alloxan induced hyperglycemic male mice serve as another example of how the abundance of flavonoids and saponins in the ethyl-acetate fraction strongly correlated with hypoglycaemic actions. According to the study, the phenolic compounds (flavonoids and saponins) in both fractions operate on several molecular targets and regulate various signaling pathways in pancreatic beta-cells, modulating the pancreatic cells' ability to regenerate and resulting in hypoglycemic activity. The stimulation of cells of pancreatic islets by the activities of the phenolics found in the ethyl-acetate fraction may be the cause of the hypoglycemic activity seen in the hyperglycemic animals. This conclusion is backed by the idea that any chemical component or secondary metabolite of a plant that might influence how much insulin is secreted from pancreatic cells may be able to prevent and treat diabetes mellitus. The ethyl-acetate fraction of *Mallotus oppostifollous* has been proposed as a candidate for a lead compound with antioxidant and antidiabetic activities by lowering blood glucose level [20]. The crude extract and fractions of *Mallotus oppostifollous* have been reported for antioxidant activity and demonstrated a significant (P=0.05) antioxidant potency of the fraction. These findings are supported by findings who reported that the presence of flavonoids, and saponins in plant drugs exhibited hypoglycemic activity in streptozotocin induced diabetic male wistar rats. This was because of their effects on a number of molecular targets that control pathways in pancreatic -cells, hepatocytes, adipocytes, and skeletal myofibers. Consequently, the flavonoids in the *M.* ethyl-acetate fraction. It is possible that *M. oppostifollous* fractions will have a significant impact on pancreatic beta-cells, causing them to proliferate, regenerate, and secrete.

Conclusion

The findings from these studies, therefore, implied that the ethyl-acetate and butanol fractions of *Mallotus oppostifollous* contained compounds that be harnessed to manage diabetes. Therefore, we draw the conclusion that more research must be done to determine the active molecule (or compounds) of the plant with the potential to treat diabetes.

References

1. Brownlee, Michael. "Biochemistry and molecular cell biology of diabetic complications." *Nature* 414 (2001): 813-820.
2. Stevens, Martin J. "Redox-based mechanisms in diabetes." *Antioxid Redox Signal* 7 (2005): 1483-1485.
3. Sreekutty MS, and S Mini. "Ensete superbum ameliorates renal dysfunction in experimental diabetes mellitus." *Iran J Basic Med Sci* 19 (2016): 111.
4. Nwaehujor, Chinaka O, Maxwell I Ezeja, Nkeiruka E Udeh, and Dozie N Okoye, et al. "Anti-inflammatory and anti-oxidant activities of Mallotus oppositifolius (Geisel) methanol leaf extracts." *Arab J Chem* 7 (2014): 805-810.
5. Fabricant, Daniel S, and Norman R Farnsworth. "The value of plants used in traditional medicine for drug discovery." *Environ Health Perspect* 109 (2001): 69-75.
6. Adekunle AA, and AM Ikumapayi. "Antifungal property and phytochemical screening of the crude extracts of Funtumia elastica and Mallotus oppositifolius." *West Indian Med J* 55 (2006): 219.
7. Rahimi-Madiseh, Mohammad, Afsaneh Malekpour-Tehrani, Mahmoud Bahmani, and Mahmoud Rafieian-Kopaei, et al. "The research and development on the antioxidants in prevention of diabetic complications." *Asian Pac J Trop Med* 9 (2016): 825-831.
8. Onyeka, Ifeanyi Peter, MO Aziakpono, CU Morikwe, and Onyebuchi Hillary Nwafor, et al. "Physicochemical and anti-diabetic effect of the crude leaf extract of Mallotus oppostifollous (Euphorbiaceae) in alloxan induced diabetic mice." *J Bioanal Biomed* 13 (2021): 279-286.
9. Dehghan, Hossein, Yaghoub Sarrafi, and Peyman Salehi. "Antioxidant and antidiabetic activities of 11 herbal plants from Hyrcania region, Iran." *J Food Drug Anal* 24 (2016): 179-188.
10. Sekhon-Loodu, Satvir, and HP Vasantha Rupasinghe. "Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants." *Front Nutr* 6 (2019): 53.
11. Patel DK, R Kumar, D Laloo, and S Hemalatha, et al. "Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity." *Asian Pac J Trop Biomed* 2 (2012): 411-420.
12. Lobo, Vijaya, Avinash Patil, A Phatak, and Naresh Chandra, et al. "Free radicals, antioxidants and functional foods: Impact on human health." *Pharmacogn Rev* 4 (2010): 118.
13. Ingle, Krishnananda P, Amit G Deshmukh, Dipika A Padole, and Mahendra S Dudhare, et al. "Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts." *J Pharmacogn Phytochem* 6 (2017): 32-36.
14. Sasidharan, Sreenivasan, Y Chen, D Saravanan, and KM Sundram, et al. "Extraction, isolation and characterization of bioactive compounds from plants' extracts." *Afr J Tradit Complement Altern Med* 8 (2011).
15. Macdonald Ighodaro, Osasenaga, Abiola Mohammed Adeosun, and Oluseyi Adeboye Akinloye. "Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies." *Medicina* 53 (2017): 365-374.
16. Lartey NL, H Asare-Anane, EK Ofori, and S Antwi, et al. "Antidiabetic activity of aqueous stem bark extract of Annickia polycarpa in alloxan-induced diabetic mice." *J Tradit Complement Med* 11 (2021): 109-116.
17. Slaoui, Mohamed, and Laurence Fiette. "Histopathology procedures: from tissue sampling to histopathological evaluation." *Methods Mol Biol* 691 (2011): 69-82.
18. Sikarwar, Mukesh S, and MB Patil. "Antidiabetic activity of Crateva nurvala stem bark extracts in alloxan-induced diabetic rats." *J Pharm Bioallied Sci* 2 (2010): 18.
19. Sparg SG, ME Light, and J van Staden. "Biological activities and distribution of plant saponins." *J Ethnopharmacol* 94 (2004): 219-243.
20. Krishnakumar K, KT Augusti, and PL Vijaymmal. "Hypoglycaemic and anti-oxidant activity of Salacia oblonga Wall. Extract in Streptezotocin-induced diabetic rats." *Indian J Physiol Pharmacol* 43 (1999): 510-514.

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